1 GCCGTCACTCCCTCCCGTCATCGATAACATCCTGTCCAAGATCGAGAACGAGTACG
1▶ AlaVal Thr Pro Ser Val IleAspAsnIleLeuSer LysIleGluAsnGluTyr G

56 AGGTGCTGTACCTGAAGCCGCTGGCAGGGGTCTACCGGAGCCTGAAGAAGCAG
19▶ luVal LeuTyr LeuLysProLeuAlaGlyVal Tyr ArgSer L uLysLysGln

109 CTGGAGAACAACGTGATGACCTTCAACGTGAACGTGAAGGATATCCTGAACAGC
37▶ LeuGluAsnAsnValMetThr PheAsnValAsnVal LysAspIleLeuAsnSer

163 CGGTTCAACAAGCGGGAGAACTTCAAGAACGTGCTGGAGAGCGATCTGATCCC
55▶ ArgPheAsnLysArgGluAsnPheLysAsnVal LeuGluSerAspLeuIlePr

216 CTACAAGGATCTGACCAGCAGCAACTACGTGGTCAAGGATCCCTACAAGTTCC
72▶ oTyr LysAspLeuThr Ser SerAsnTyr Val ValLysAspProTyr LysPheL

269 TGAACAAGGAGAAGAGAGATAAGTTCCTGAGCAGTTACAACTACATCAAGGATAG
90▶ euAsnLysGluLysArgAspLysPheLeuSer Ser TyrAsnTyr IleLysAspSe

324 CATTGATACCGATATCAACTTCGCCAACGATGTCCTGGGATACTACAAGATCCT
108▶ r IleAspThrAspIleAsnPheAlaAsnAspVal LeuGlyTyr Tyr LysIleLe

378 GTCCGAGAAGTACAAGAGCGATCTGGATTCAATCAAGAAGTACATCAACGATAA
126▶ uSer GluLysTyr Lys SerAspLeuAspSer IleLysLysTyr IleAsnAspLy

432 GCAGGGAGAGAACGAGAAGTACCTGCCCTTCCTGAACAACATCGAGACCCTGTA
144▶ sGlnGlyGluAsnGluLysTyrLeuProPhe LeuAsnAsnIleGluThr LeuTy

486 CAAGACCGTCAACGATAAGATTGATCTGTTCGTGATCCACCTGGAGGCCAAGGT
162▶ r LysThr ValAsnAspLysIleAspLeuPheVal IleHis LeuGluAlaLysVa

NdeI

540 CCTGAACTACACATATGAGAAGAGCAACGTGGAGGTCAAGATCAAGGAGCTGAA
180▶ lLeuAsnTyr Thr TyrGluLys SerAsnVal GluVal LysIleLysGluLeuAs

594 TTACCTGAAGACCATCCAGGATAAGCTGGCCGATTTCAAGAAGAACAACAACTT
198▶ nTyrLeuLysThr IleGlnAspLysLeuAlaAspPheLysLysAsnAsnAsnPh

648 CGTCGGGATCGCCGATCTGAGCACCGATTACAACCACAACAACCTGCTGACCAA
216▶ eVal GlyIleAlaAspLeu Ser ThrAspTyr AsnHisAsnAsnLeu LeuThr Ly

702 GTTCCTGAGCACCGGTATGGTCTTCGAAAACCTGGCCAAGACCGTCCTGAGCAA
234▶ sPheLeuSer Thr GlyMetVal PheGluAsnLeuAlaLysThrVal Leu SerAs

756 CCTGCTGGATGGGAACCTGCAGGGGATGCTGAACATCAGCCAGCACCAGTGTGT
252▶ nLeuLeuAspGlyAsnLeuGlnGlyMet LeuAsnIle Ser GlnHisGlnCys Va

810 GAAGAAGCAGTGTCCCCAGAACAGCGGGTGTTTCAGACACCTGGATGAGAGAGA
270▶ lLysLysGlnCysProGlnAsnSer GlyCysPheArgHis LeuAspGluArgGl

864 GGAGTGTAAGTGTCTGCTGAACTACAAGCAGGAAGGTGATAAGTGTGTGGAAAAC
288▶ uGluCysLysCysLeu LeuAsnTyrLysGlnGluGlyAspLysCysVal GluAsn

919 CCCAATCCTACTTGTAACGAGAACAATGGTGGATGTGATGCCGATGCCAAGTGTACCG
307▶ ProAsnProThr CysAsnGluAsnAsnGlyGlyCysAspAlaAspAlaLysCysThr G

977 AGGAGGATTCAGGGAGCAACGGGAAGAAGATCACCTGTGAGTGTACCAAGCCTGATT
326▶ luGluAspSer Gly SerAsnGlyLysLysIleThr CysGluCysThr LysProAspS

1034 CTTATCCACTGTTCGATGGTATCTTCTGTAGT
345▶ er TyrProLeuPheAspGlyIlePheCys Ser

FIG. 1

```
   1   GCAGTAACTCCTTCCGTAATTGATAACATACTTTCTAAAATTGAAAATGAATA
   1▶ AlaValThrProSerValIleAspAsnIleLeuSerLysIleGluAsnGluTyrG
                          EcoNI (73)
  56   AGGTTTTATATTTAAAACCTTTAGCAGGTGTTTATAGAAGTTTAAAAAAACAATT
  19▶ luValLeuTyrLeuLysProLeuAlaGlyValTyrArgSerLeuLysLysGlnLe
 111   AGAAAAATAACGTTATGACATTTAATGTTAATGTTAAGGATATTTTAAATTCACGA
  37▶ uGluAsnAsnValMetThrPheAsnValAsnValLysAspIleLeuAsnSerArg
 166   TTTAATAAACGTGAAAATTTCAAAAATGTTTTAGAATCAGATTTAATTCCATATA
  56▶ PheAsnLysArgGluAsnPheLysAsnValLeuGluSerAspLeuIleProTyrL
 221   AAGATTTAACATCAAGTAATTATGTTGTCAAAGATCCATATAAATTTCTTAATAA
  74▶ ysAspLeuThrSerSerAsnTyrValValLysAspProTyrLysPheLeuAsnLy
 276   AGAAAAAAGAGATAAATTCTTAAGCAGTTATAATTATATTAAGGATTCAATAGAT
  92▶ sGluLysArgAspLysPheLeuSerSerTyrAsnTyrIleLysAspSerIleAsp
 331   ACGGATATAAATTTTGCAAATGATGTTCTTGGATATTATAAAATATTATCCGAAA
 111▶ ThrAspIleAsnPheAlaAsnAspValLeuGlyTyrTyrLysIleLeuSerGluL
 386   AATATAAATCAGATTTAGATTCAATTAAAAAATATATCAACGACAAACAAGGTGA
 129▶ ysTyrLysSerAspLeuAspSerIleLysLysTyrIleAsnAspLysGlnGlyGl
 441   AAATGAGAAATACCTTCCCTTTTTAAACAATATTGAGACCTTATATAAAACAGTT
 147▶ uAsnGluLysTyrLeuProPheLeuAsnAsnIleGluThrLeuTyrLysThrVal
 496   AATGATAAAATTGATTTATTTGTAATTCATTTAGAAGCAAAAGTTCTAAATTATA
 166▶ AsnAspLysIleAspLeuPheValIleHisLeuGluAlaLysValLeuAsnTyrT
 551   CATATGAGAAATCAAACGTAGAAGTTAAAATAAAAGAACTTAATTACTTAAAAAC
 184▶ hrTyrGluLysSerAsnValGluValLysIleLysGluLeuAsnTyrLeuLysTh
 606   AATTCAAGACAAATTGGCAGATTTTAAAAAAAATAACAATTTCGTTGGAATTGCT
 202▶ rIleGlnAspLysLeuAlaAspPheLysLysAsnAsnAsnPheValGlyIleAla
 661   GATTTATCAACAGATTATAACCATAATAACTTATTGACAAAGTTCCTTAGTACAG
 221▶ AspLeuSerThrAspTyrAsnHisAsnAsnLeuLeuThrLysPheLeuSerThrG
 716   GTATGGTTTTTGAAAATCTTGCTAAAACCGTTTTATCTAATTTACTTGATGGAAA
 239▶ lyMetValPheGluAsnLeuAlaLysThrValLeuSerAsnLeuLeuAspGlyAs
 771   CTTGCAAGGTATGTTAAACATTTCACAACACCAATGCGTAAAAAAACAATGTCCA
 257▶ nLeuGlnGlyMetLeuAsnIleSerGlnHisGlnCysValLysLysGlnCysPro
 826   CAAAATTCTGGATGTTTCAGACATTTAGATGAAAGAGAAGAATGTAAATGTTTAT
 276▶ GlnAsnSerGlyCysPheArgHisLeuAspGluArgGluGluCysLysCysLeuL
 881   TAAATTACAAACAAGAAGGTGATAAATGTGTTGAAAATCCAAATCCTACTTGTAA
 294▶ euAsnTyrLysGlnGluGlyAspLysCysValGluAsnProAsnProThrCysAs
 936   CGAAAATAATGGTGGATGTGATGCAGATGCCAAATGTACCGAAGAAGATTCAGGT
 312▶ nGluAsnAsnGlyGlyCysAspAlaAspAlaLysCysThrGluGluAspSerGly
 991   AGCAACGGAAAGAAAATCACATGTGAATGTACTAAACCTGATTCTTATCCACTTT
 331▶ SerAsnGlyLysLysIleThrCysGluCysThrLysProAspSerTyrProLeuP
                      PstI   (1059)
1046   TCGATGGTATTTTCTGCAGTCACCACCACCACCACCACTAACT
 349▶ heAspGlyIlePheCysSerHisHisHisHisHisHis•••
```

FIG. 2

Codon	AA	goat b-casei	goat K-casein	MSP wt	Edited MSP	mouse b-casei	mouse a-casei	mouse g-casein	mouse e-casei
TTT	Phe	5	4	8	0	4	8	3	4
TTC	Phe	4	3	7	15	4	6	7	1
TTA	Leu	0	2	25	0	0	0	0	0
TTG	Leu	0	2	3	0	0	0	0	1
TCT	Ser	5	1	4	1	13	5	7	5
TCC	Ser	2	2	2	3	6	14	8	2
TCA	Ser	1	4	10	1	1	3	2	0
TCG	Ser	0	1	0	0	0	0	0	0
TAT	Tyr	2	7	17	2	1	3	2	1
TAC	Tyr	1	2	3	18	2	6	6	7
TAA	***	1	2	0	0	1	0	1	0
TAG	***	0	0	0	0	0	0	0	0
TGT	Cys	1	1	10	12	0	0	1	0
TGC	Cys	0	2	2	0	2	2	2	1
TGA	***	0	0	0	0	0	1	0	1
TGG	Trp	1	1	0	0	0	2	2	2
CTT	Leu	9	1	9	0	16	9	3	3
CTC	Leu	5	2	0	0	7	8	0	1
CTA	Leu	1	2	1	0	1	2	1	0
CTG	Leu	11	5	0	38	10	17	4	1
CCT	Pro	17	6	4	2	8	6	3	0
CCC	Pro	12	0	1	6	8	6	6	4
CCA	Pro	3	13	5	1	5	6	2	2
CCG	Pro	1	1	0	1	0	0	0	1
CAT	His	0	1	3	0	2	6	2	1
CAC	His	5	3	1	4	4	0	3	0
CAA	Gln	5	9	9	0	9	21	9	7
CAG	Gln	16	6	0	9	21	32	12	8
CGT	Arg	0	1	1	0	0	0	0	0
CGC	Arg	0	0	0	0	1	0	0	0
CGA	Arg	0	0	1	0	0	0	0	1
CGG	Arg	1	0	0	3	0	0	0	0
ATT	Ile	4	5	13	0	3	4	3	4
ATC	Ile	6	3	2	20	7	5	8	5
ATA	Ile	1	3	5	0	1	0	2	0
ATG	Met	7	3	3	3	4	12	2	13
ACT	Thr	7	6	3	2	6	5	1	4
ACC	Thr	2	7	3	13	4	4	4	4
ACA	Thr	2	4	9	1	1	1	2	0
ACG	Thr	0	0	1	0	0	0	2	0
AAT	Asn	2	6	29	3	4	6	3	1
AAC	Asn	2	3	12	38	4	9	4	6
AAA	Lys	7	6	38	0	6	7	3	5
AAG	Lys	6	4	4	42	3	6	13	7
AGT	Ser	2	6	5	2	3	6	6	5
AGC	Ser	5	0	2	16	2	6	6	3
AGA	Arg	2	2	4	3	1	8	1	1
AGG	Arg	0	2	0	0	0	0	0	1
GTT	Val	5	6	15	0	7	4	2	3
GTC	Val	8	2	1	11	7	3	3	0
GTA	Val	2	2	5	0	2	4	1	3
GTG	Val	8	4	0	10	6	3	5	3
GCT	Ala	1	3	2	0	8	17	4	2
GCC	Ala	4	7	1	8	6	3	3	3
GCA	Ala	3	7	6	1	4	13	1	1
GCG	Ala	0	1	0	0	0	0	0	0
GAT	Asp	4	5	25	27	3	6	4	2
GAC	Asp	0	2	2	0	1	2	1	3
GAA	Glu	10	6	21	3	6	12	9	6
GAG	Glu	9	5	4	22	5	5	5	5
GGT	Gly	2	1	8	4	0	0	0	0
GGC	Gly	0	0	0	0	0	0	0	0
GGA	Gly	2	1	6	3	1	0	1	0
GGG	Gly	1	0	0	7	1	0	0	0

FIG. 3A

Codon	AA	MSP wt	Edited MSP	MSP wt	Edited MSP	E.coli	Human
TTT	Phe	8	0	0.53	0	0.5	0.35
TTC	Phe	7	15	0.47	1	0.5	0.65
TTA	Leu	25	0	0.66	0	0.11	0.05
TTG	Leu	3	0	0.08	0	0.11	0.09
TCT	Ser	4	1	0.17	0.04	0.27	0.17
TCC	Ser	2	3	0.09	0.13	0.21	0.26
TCA	Ser	10	1	0.43	0.04	0.13	0.11
TCG	Ser	0	0	0	0	0.14	0.07
TAT	Tyr	17	2	0.85	0.1	0.54	0.47
TAC	Tyr	3	18	0.15	0.9	0.46	0.53
TAA	***	0	0				
TAG	***	0	0				
TGT	Cys	10	12	0.83	1	0.45	0.3
TGC	Cys	2	0	0.17	0	0.55	0.7
TGA	***	0	0				
TGG	Trp	0	0	0	0	1	1
CTT	Leu	9	0	0.24	0	0.12	0.11
CTC	Leu	0	0	0	0	0.12	0.22
CTA	Leu	1	0	0.02	0	0.03	0.07
CTG	Leu	0	38	0	1	0.72	0.46
CCT	Pro	4	2	0.4	0.2	0.14	0.24
CCC	Pro	1	6	0.1	0.6	0.11	0.41
CCA	Pro	5	1	0.5	0.1	0.2	0.24
CCG	Pro	0	1	0	0.1	0.54	0.11
CAT	His	3	0	0.75	0	0.64	0.42
CAC	His	1	4	0.25	1	0.36	0.58
CAA	Gln	9	0	1	0	0.31	0.26
CAG	Gln	0	9	0	1	0.69	0.74
CGT	Arg	1	0	0.17	0	0.46	0.09
CGC	Arg	0	0	0	0	0.32	0.19
CGA	Arg	1	0	0.17	0	0.05	0.1
CGG	Arg	0	3	0	0.5	0.06	0.15
ATT	Ile	13	0	0.65	0	0.39	0.23
ATC	Ile	2	20	0.1	1	0.52	0.64
ATA	Ile	5	0	0.25	0	0.08	0.13
ATG	Met	3	3	1	1	1	1
ACT	Thr	3	2	0.19	0.13	0.36	0.2
ACC	Thr	3	13	0.19	0.81	0.38	0.47
ACA	Thr	9	1	0.56	0.06	0.09	0.21
ACG	Thr	1	0	0.06	0	0.17	0.12
AAT	Asn	29	3	0.71	0.07	0.29	0.34
AAC	Asn	12	38	0.29	0.93	0.71	0.66
AAA	Lys	38	0	0.9	0	0.72	0.45
AAG	Lys	4	42	0.1	1	0.28	0.55
AGT	Ser	5	2	0.21	0.09	0.11	0.11
AGC	Ser	2	16	0.09	0.7	0.14	0.29
AGA	Arg	4	3	0.67	0.5	0.08	0.24
AGG	Arg	0	0	0	0	0.03	0.23
GTT	Val	15	0	0.71	0	0.37	0.13
GTC	Val	1	11	0.05	0.52	0.12	0.27
GTA	Val	5	0	0.24	0	0.28	0.09
GTG	Val	0	10	0	0.48	0.23	0.5
GCT	Ala	2	0	0.22	0	0.33	0.31
GCC	Ala	1	8	0.11	0.89	0.18	0.4
GCA	Ala	6	1	0.67	0.11	0.28	0.17
GCG	Ala	0	0	0	0	0.21	0.12
GAT	Asp	25	27	0.93	1	0.48	0.38
GAC	Asp	2	0	0.07	0	0.52	0.62
GAA	Glu	21	3	0.84	0.12	0.67	0.4
GAG	Glu	4	22	0.16	0.88	0.33	0.6
GGT	Gly	8	4	0.57	0.29	0.46	0.15
GGC	Gly	0	0	0	0	0.4	0.44
GGA	Gly	6	3	0.43	0.21	0.06	0.17
GGG	Gly	0	7	0	0.5	0.08	0.24

FIG. 3B

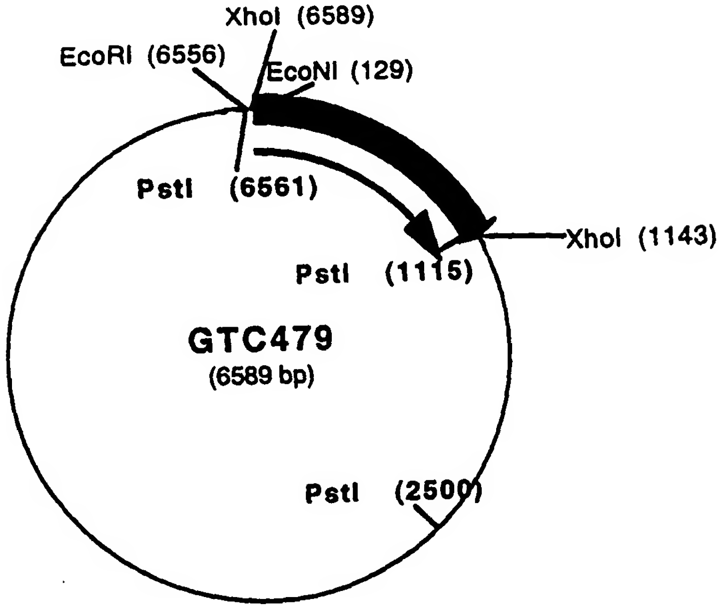

XhoI (6589)
EcoRI (6556)
EcoNI (129)
PstI (6561)
XhoI (1143)
PstI (1115)
GTC479
(6589 bp)
PstI (2500)

FIG. 4A

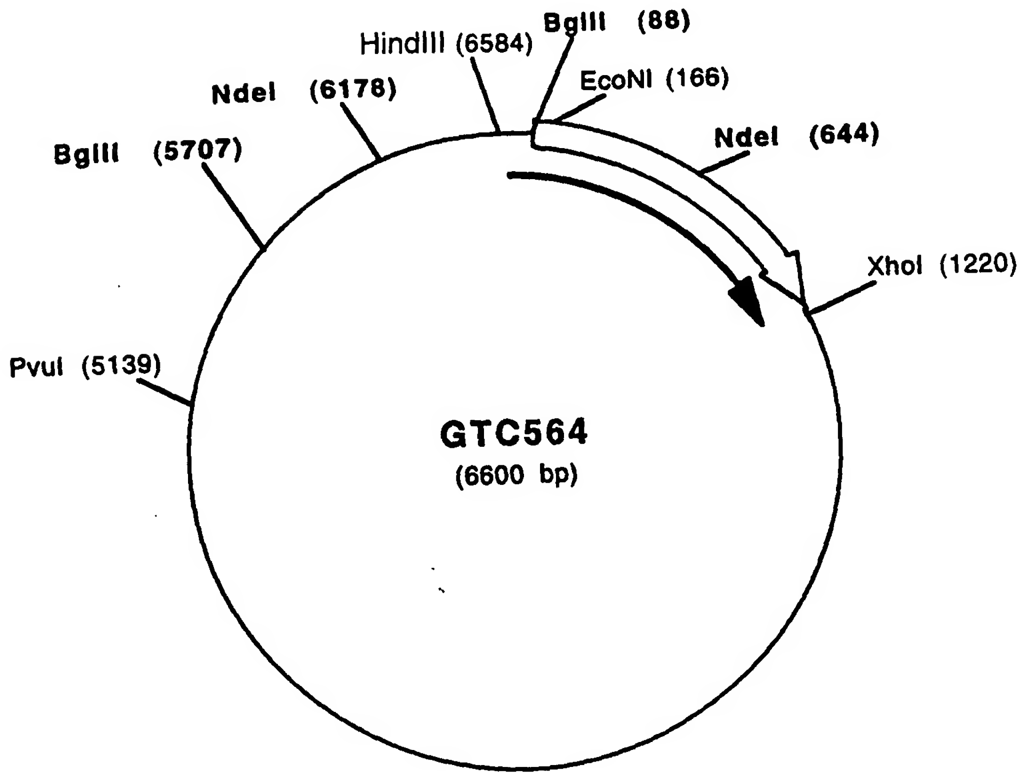

HindIII (6584)
BglII (88)
EcoNI (166)
NdeI (6178)
BglII (5707)
NdeI (644)
XhoI (1220)
PvuI (5139)
GTC564
(6600 bp)

FIG. 4B

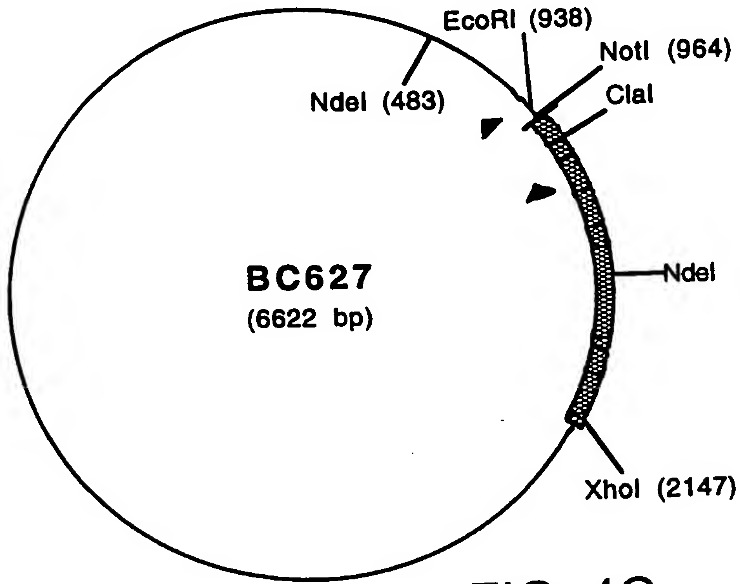


FIG. 4C

Oligos used:

OT1:
TCG ACG AGA GCC ATG AAG GTC CTC ATC CTT GCC TGT CTG GTG GCT CTG GCC ATT GCA AGA GAG CAG GAA GAA CTC AAT GTA GTC GGT A,

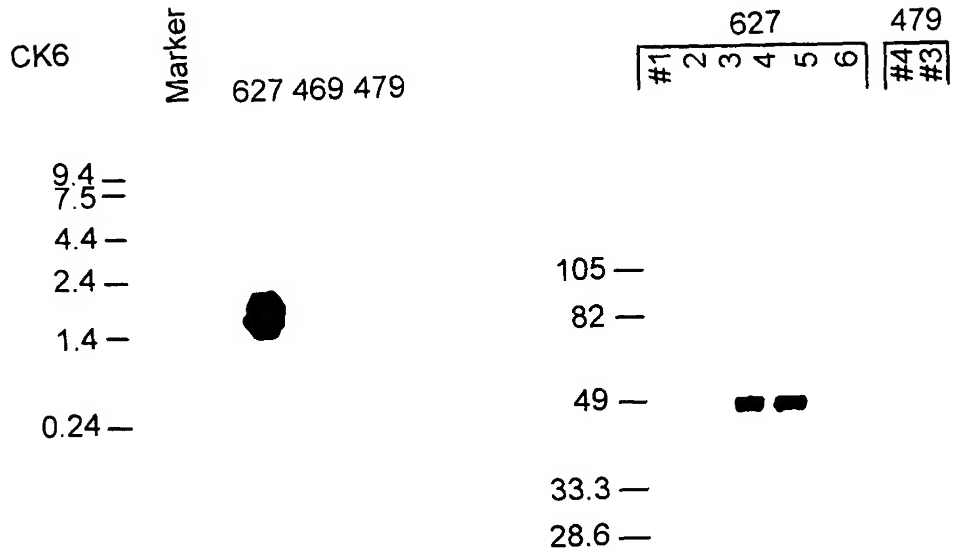
OT2:
GAT CTA CCG ACT ACA TTG AGT TCT TCC TGC TCT CTT GCA ATG GCC AGA GCC ACC AGA CAG GCA AGG ATG AGG ACC TTC ATG GCT CTC G,

MSP1:
AATAGATCTGCAGTAACTCCTTCCGTAATTG,

MSP2:
AATTCTCGAGTTAGTGGTGGTGGTGGTGGTGACTGCAGAAATACCATC

MSP8:
TAACTCGAGCGAACCATGAAGGTCCTCATCCTTGCCTGTCTGGTGGCTCTGG
CCATTGCA

FIG. 6



PANEL A

PANEL B

FIG. 5

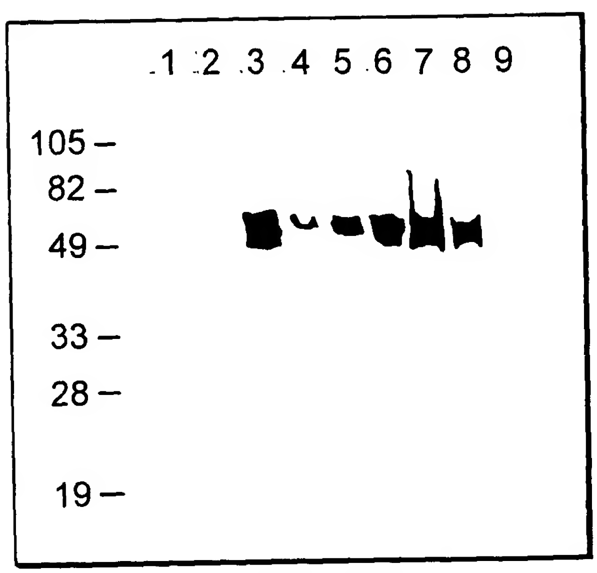


FIG. 10

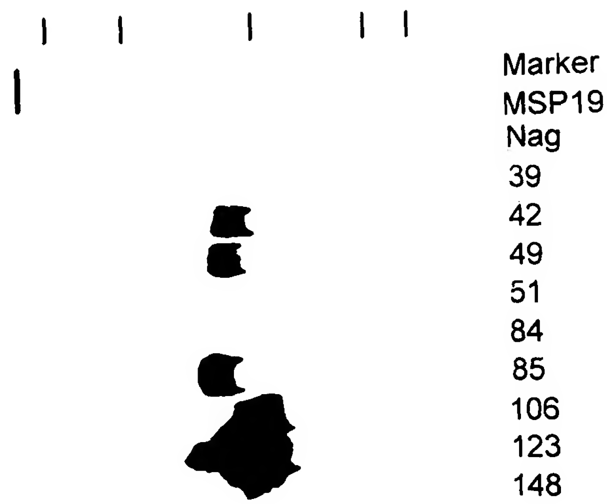


FIG. 13

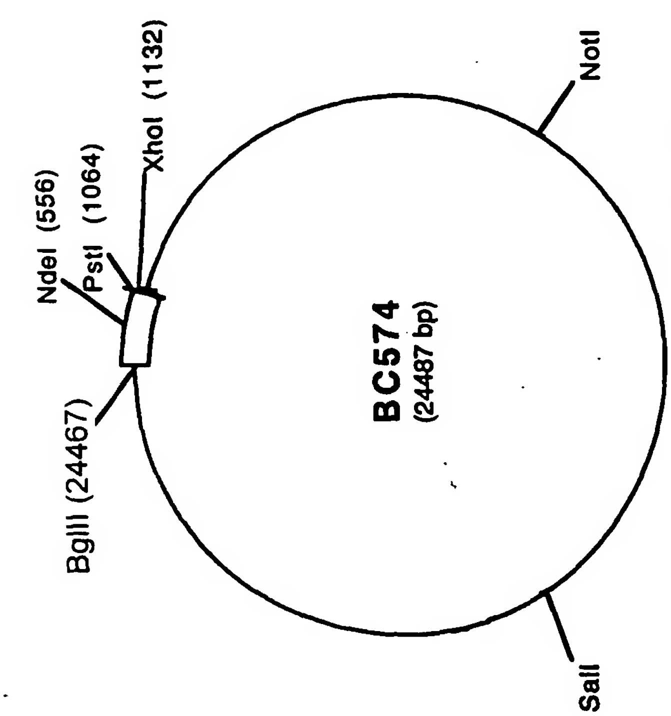


FIG. 7

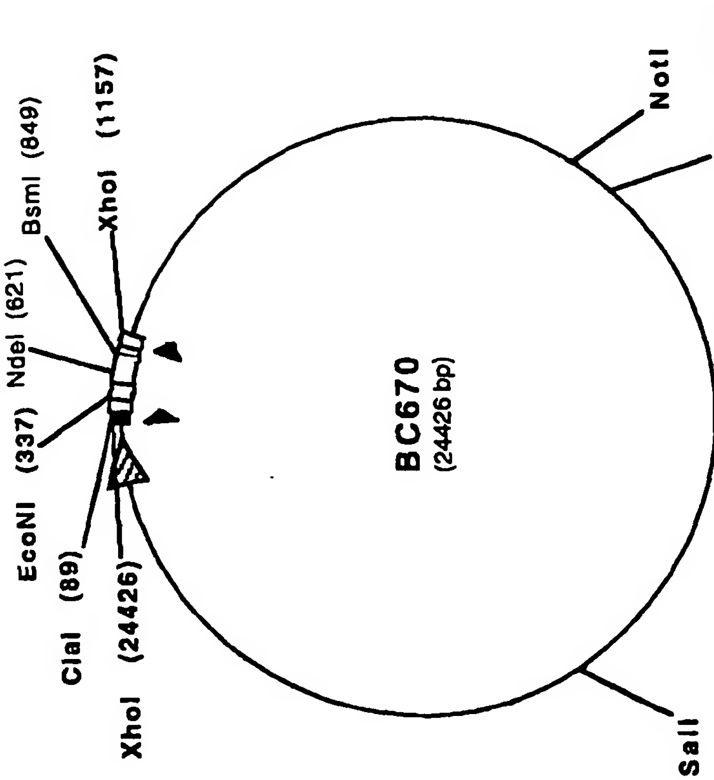


FIG. 8

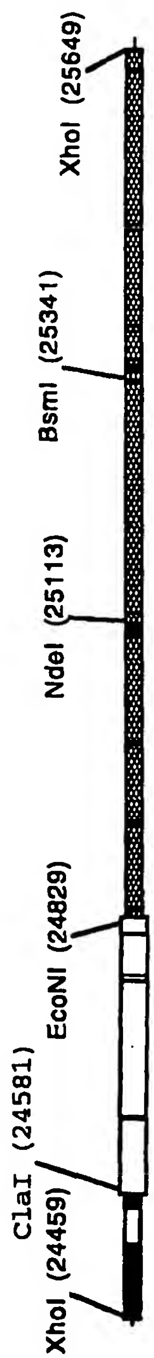


Diagram of BC620

FIG. 9

26 ATGAAGGTCCTCATAATTGCCTGTCTGGTGGCTCTGGCCATTGCAGCCGTCACTCCCTCCGTCATCGATAAC

1▸ M K V L I I A C L V A L A I A A V T P S V I D N

98 ATCCTGTCCAAGATCGAGAACGAGTACGAGGTGCTGTACCTGAAGCCCCTGGCAGGAGTCTACAGGAGCCT

25▸ I L S K I E N E Y E V L Y L K P L A G V Y R S L

169 GAAGAAGCAGCTGGAGAACAACGTGATGACCTTCAACGTGAACGTGAAGGATATCCTGAACAGCAGGTTCAA

48▸ K K Q L E N N V M T F N V N V K D I L N S R F N

241 CAAGAGGGAGAACTTCAAGAACGTGCTGGAGAGCGATCTGATCCCCTACAAGGATCTGACCAGCAGCAACTA

72▸ K R E N F K N V L E S D L I P Y K D L T S S N Y

EcoNI (337)

313 CGTGGTCAAAGATCCCTACAAGTTCCTGAACAAGGAGAAGAGAGATAAGTTCCTGAGCAGTTACAATTACAT

96▸ V V K D P Y K F L N K E K R D K F L S S Y N Y I

385 CAAGGATAGCATTGACACCGATATCAACTTCGCCAACGATGTCCTGGGATACTACAAGATCCTGTCCGAGAA

120▸ K D S I D T D I N F A N D V L G Y Y K I L S E K

457 GTACAAGAGCGATCTGGATAGCATCAAGAAGTACATCAACGATAAGCAGGGAGAGAACGAGAAGTACCTGCC

144▸ Y K S D L D S I K K Y I N D K Q G E N E K Y L P

529 CTTCCTGAACAACATCGAGACCCTGTACAAGACCGTCAACGATAAGATTGATCTGTTCGTGATCCACCTGGA

168▸ F L N. N I E T L Y K T V N D K I D L F V I H L E

NdeI (621)

601 GGCCAAGGTCCTGCAGTACACATATGAGAAGAGCAACGTGGAGGTCAAGATCAAGGAGCTGAATTACCTGAA

192▸ A K V L Q Y T Y E K S N V E V K I K E L N Y L K

673 GACCATCCAGGATAAGCTGGCCGATTTCAAGAAGAACAACAACTTCGTCGGAATCGCCGATCTGAGCACCGA

216▸ T I Q D K L A D F K K N N N F V G I A D L S T D

745 TTACAACCACAACAACCTGCTGACCAAGTTCCTGAGCACCGGAATGGTCTTCGAAAACCTGGCCAAGACCGT

240▸ Y N H N N L L T K F L S T G M V F E N L A K T V

BsmI (849)

817 CCTGAGCAACCTGCTGGATGGAAACCTGCAGGGAATGCTGCAGATCAGCCAGCACCAGTGTGTGAAGAAGC

264▸ L S N L L D G N L Q G M L Q I S Q H Q C V K K

888 AGTGTCCCCAGAACAGCGGATGCTTCAGACACCTGGATGAGAGGGAGGAGTGCAAGTGCCTGCTGAACTA

288▸ Q C P Q N S G C F R H L D E R E E C K C L L N Y

958 CAAGCAGGAAGGAGATAAGTGTGTGGAAAACCCCAATCCTACTTGTAACGAGAACAATGGAGGATGCGATG

311▸ K Q E G D K C V E N P N P T C N E N N G G C D

1029 CCGATGCCAAGTGTACCGAGGAGGATTCAGGAAGCAACGGAAAGAAGATCACCTGCGAGTGTACCAAGCCT

335▸ A D A K C T E E D S G S N G K K I T C E C T K P

1100 GATTCTTATCCACTGTTCGATGGtATtTTCTGCAGTCACCACCACCACCACCACTAACTCGAGGAT

XhoI (1157)

359▸ D S Y P L F D G I F C S H H H H H H • L E D

FIG. 11

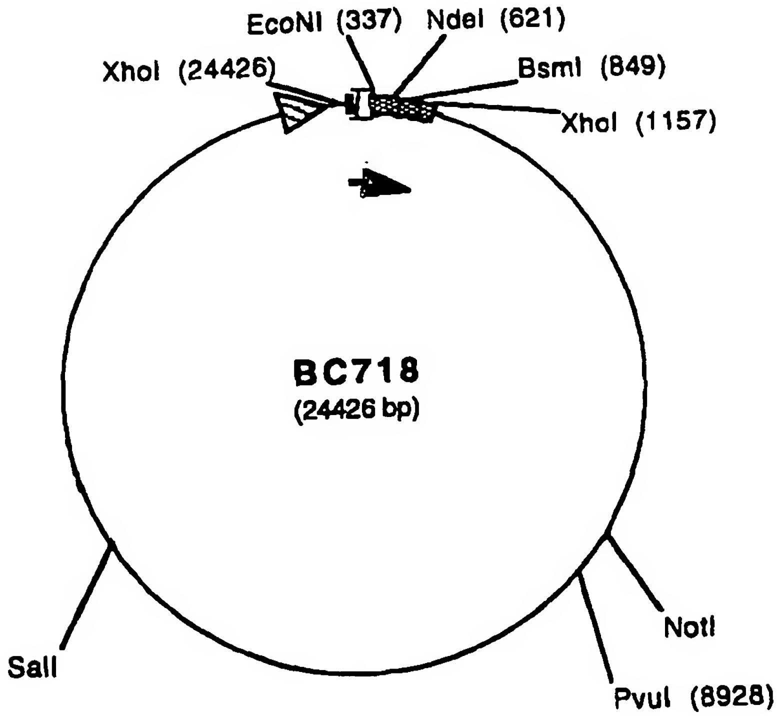

EcoNI (337)
NdeI (621)
XhoI (24426)
BsmI (849)
XhoI (1157)
BC718
(24426 bp)
SalI
NotI
PvuI (8928)

FIG. 12